REVIEW

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Glial and neuronal control of brain blood flow

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Blood flow in the brain is regulated by neurons and astrocytes. Knowledge of how these cells control blood flow is crucial for understanding how neural computation is powered, for interpreting functional imaging scans of brains, and for developing treatments for neurological disorders. It is now recognized that neurotransmitter–mediated signalling has a key role in regulating cerebral blood flow, that much of this control is mediated by astrocytes, that oxygen modulates blood flow regulation, and that blood flow may be controlled by capillaries as well as by arterioles. These conceptual shifts in our understanding of cerebral blood flow control have important implications for the development of new therapeutic approaches.

The human brain comprises only 2% of the body's mass, but it consumes 20% of the energy that is produced when the body is in a resting state. This high consumption of energy is crucial for the normal functioning of the brain. The energy is mostly used to reverse the ion influxes that underlie synaptic potentials and action potentials[1] (Fig. 1a). If there is an inadequate supply of blood glucose and oxygen to a region of the brain, then neurons and glia become injured or die. This occurs in disorders such as ischaemic stroke, vasospasm after sub-arachnoid haemorrhage, the secondary ischaemia that follows spinal-cord injury, and cerebral palsy after perinatal asphyxia. To sustain neuronal function, the brain has evolved 'neurovascular coupling' mechanisms to increase the flow of blood to regions in which neurons are active, a response termed functional hyperaemia. Different information coding strategies and neural algorithms require different increases in blood flow, depending on the extent to which they consume energy. An understanding of the mechanisms that generate functional hyperaemia is a prerequisite for developing therapies to correct defects in blood flow control that occur after disorders such as stroke[2], hypertension[3], spinal-cord injury[4] and Alzheimer's disease[3].

Concepts of how neuronal activity controls the vascular supply of glucose and O_2 are changing rapidly. Traditionally, it was thought that active neurons generate a metabolic signal (a fall in O_2 or glucose concentration, or a rise in carbon dioxide concentration), which triggers an increase in blood flow. This idea has recently been superseded, following the discovery that neurotransmitter-mediated signalling, particularly by glutamate, has a major role in regulating cerebral blood flow, and that much of this control is mediated by astrocytes. Glutamate-mediated signalling leads to the release of nitric oxide from neurons and of arachidonic acid derivatives from astrocytes (and possibly from neurons). These molecules can either increase or decrease blood flow, depending on the local O_2 concentration, but how this switch occurs is debated. Furthermore, the relative importance of the different glutamate-released messengers varies between brain regions. Even the dogma that cerebral blood flow is controlled solely by arterioles has been challenged, with the finding that contractile cells called pericytes can control the diameter of capillaries, and that damage to these cells contributes to the long-lasting decrease in blood flow that occurs after stroke. These major conceptual shifts, which we discuss in this Review, provide a new understanding of how the brain regulates its energy supply in response to different information processing tasks. They also underpin the interpretation of data from functional imaging experiments, and offer new opportunities for developing therapeutic approaches to a range of disorders of the central nervous system.

Neurotransmitters increase cerebral blood flow

In this Review, we focus on the control of cerebral blood flow by local neuronal activity. We pay less attention to autoregulation, which, in the face of changes in systemic blood pressure, maintains an approximately constant blood flow to the brain.

Early researchers favoured the idea that blood flow is locally controlled by a negative-feedback system in which neural activity leads to energy demand, because ATP is used to restore ion gradients after the generation of synaptic and action potentials[1]. This ATP use was thought to produce a metabolic signal that increased blood flow and therefore provided more energy (Fig. 1b). This metabolic signal could be a lack of O_2 or glucose, or the production of CO_2 (which dilates cerebral vessels by being converted to H^+ after combining with H_2O)[5].

However, manipulation of blood O_2 (refs 6, 7) and glucose[8] concentrations to test this negative-feedback hypothesis has shown that O_2 and glucose do not regulate blood flow in this way. Furthermore, during neuronal activity, the local extracellular pH initially becomes alkaline, rather than becoming acidic as would be expected if arteriolar dilation were caused by the accumulation of CO_2. This is partly because the increase in blood flow elicited by neural activity washes CO_2 away rapidly, and partly because the neuronal Ca^{2+}, H^+-ATPase pump alkalinizes the extracellular space when neural activity raises the neuronal intracellular calcium concentration ($[Ca^{2+}]_i$)[9,10]. These findings do not support a local CO_2 rise as the cause of the increased blood flow — despite the fact that exogenous CO_2 does increase blood flow. However, the 'metabolic messenger' adenosine (which is produced when ATP is hydrolysed) does contribute to functional hyperaemia, because blocking adenosine receptors reduces the increase in blood flow evoked by neuronal activity[11]. Furthermore, another metabolic messenger, lactate (which is produced when pyruvate production by glycolysis outstrips pyruvate consumption by oxidative phosphorylation), also increases

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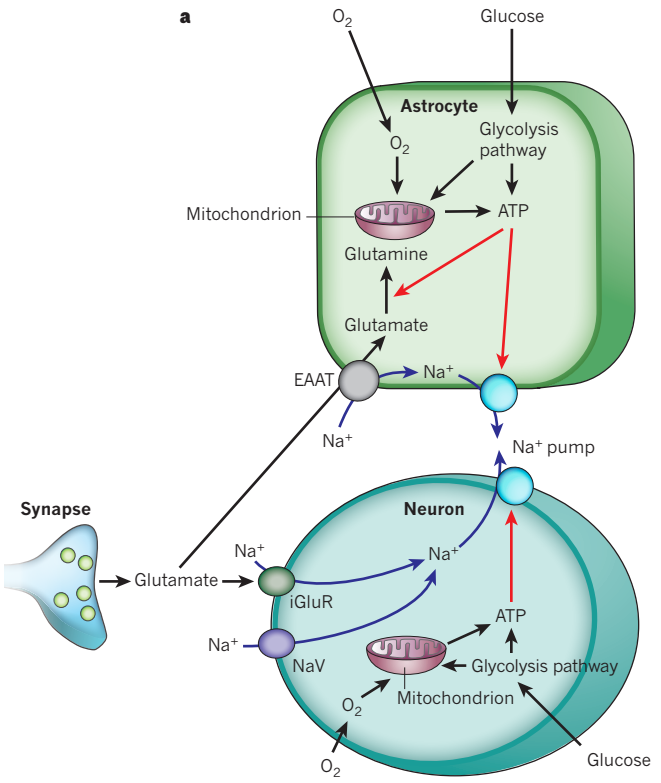

Figure 1 | Energy supply, usage and blood flow regulation in the brain. **a**, ATP is generated from glycolysis and mitochondrial oxidative phosphorylation in neurons and glia. ATP is mainly consumed (red arrows) by ion pumping in neurons, to maintain the ion gradients underlying synaptic and action potentials, following Na^+ entry (blue arrows) through ionotropic glutamate receptors (iGluR) and voltage-gated Na^+ channels (NaV). It is also used in glia for Na^+-coupled neurotransmitter uptake by excitatory amino acid transporters (EAAT) and for metabolic processing (shown for conversion of glutamate to glutamine), and on maintaining the cells' resting potentials. **b**, The negative-feedback control hypothesis for vascular energy supply, in which a fall in energy level induces an increased cerebral blood flow (CBF). **c**, The feedforward regulation hypothesis for vascular energy supply.

blood flow[12]. However, as explained below (see 'O_2 modulates neurovascular signalling'), this is mediated at least partly by a modulation of neurotransmitter-induced prostaglandin signalling to blood vessels.

More recent work has shown that control of the vascular energy supply by neural activity is largely mediated by feedforward mechanisms (Fig. 1c). In these processes, neurons either signal directly to blood vessels or activate astrocytes to release vasoactive agents onto the vessels. For both of these signalling routes, the coupling mechanisms involve neurotransmitter, particularly glutamate, signalling (Fig. 2).

One cannot distinguish between metabolic feedback and neurotransmitter feedforward mechanisms simply by blocking neurotransmitter-mediated signalling. Although neurotransmitter antagonists block the increase in blood flow elicited by neural activity[13–17], they also block the energy consumption evoked by synaptic signalling[1,18,19]. However, the roles of energy consumption and neurotransmitter-mediated signalling in regulating blood flow can be disentangled. Blocking the enzymes downstream of glutamate receptors that generate nitric oxide and arachidonic acid derivatives greatly reduces functional hyperaemia but has little effect on the energy use that is associated with neural activity[18,20].

Neuronal signalling to blood vessels

Synaptic release of glutamate activates neuronal NMDA (*N*-methyl-D-aspartate) receptors, resulting in Ca^{2+} entry into neurons and activation of neuronal nitric oxide synthase (nNOS). This releases NO, which dilates vessels[21], both in brain slices (which allow more refined mechanistic investigations, but lack intravascular pressure and blood flow that can release messengers that modulate the properties of vascular smooth muscle) and *in vivo* (Fig. 2). In the cortex, inhibition of nNOS reduces the increase in blood flow that is associated with neural activity[22], suggesting a role for NO in neurovascular coupling. However, the response is restored by the addition of NO donors, which provide a constant concentration of NO. This indicates that, although the presence of NO is required, a dynamic rise of NO concentration in response to neuronal activity does not directly mediate neuron-to-vessel signalling[23]. Instead, NO might be needed to modulate the pathways in astrocytes which dilate and constrict blood vessels (see 'NO modulates astrocyte signalling'). In the cerebellum, by contrast, NO donors do not reverse the reduction in functional hyperaemia caused by inhibiting nNOS, demonstrating that NO directly mediates a component of the response[24,25]. The activation of peptidergic interneurons can also dilate or constrict vessels in the cortex[26], but the dilations that have been observed were usually irreversible (within the 7-min recording period). In addition, it is unclear whether these effects involve peptide release and whether they reflect direct signalling from neurons to vessels or indirect signalling by way of astrocytes (see 'Astrocyte signalling to blood vessels'). Finally, the neurotransmitter GABA (γ-aminobutyric acid), acting through $GABA_A$ receptors, also mediates a component of the vasodilation produced in the cortex by basal forebrain stimulation[27], but it is unclear whether this is a direct effect on the vasculature or is mediated by an effect on neurons or astrocytes.

Astrocyte signalling to blood vessels

Astrocytes are ideally situated to function as relay cells in neurovascular communication, as was suggested more than a century ago by Ramón y Cajal. They surround synapses and thus can be stimulated by neuronal activity, whereas their endfoot processes envelop blood vessels and can signal to the smooth muscle cells that control vessel diameter.

In theory, astrocytes can increase blood flow in response to neuronal activity by releasing potassium ions from their endfeet apposed to arterioles, because modest increases in extracellular K^+ concentration (up to ~10 mM $[K^+]_o$) hyperpolarize smooth muscle cells. This occurs because a raised $[K^+]_o$ increases the conductance of smooth muscle inward rectifier K^+ channels, and this effect outweighs the positive shift of the K^+ reversal potential, E_K, produced by the raised $[K^+]_o$ and leads to more outward current flowing (because the membrane potential is more positive than E_K). This hyperpolarization reduces the influx of Ca^{2+} through voltage-gated channels and dilates the vessels[28]. The 'K^+ siphoning' hypothesis holds that K^+ released from active neurons depolarizes astrocytes, leading to K^+ efflux from astrocyte endfeet[29]. This mechanism of neurovascular coupling has been tested by directly depolarizing glial cells while monitoring blood vessel diameter[30]. Depolarization fails to dilate vessels, demonstrating that K^+ siphoning does not contribute significantly to

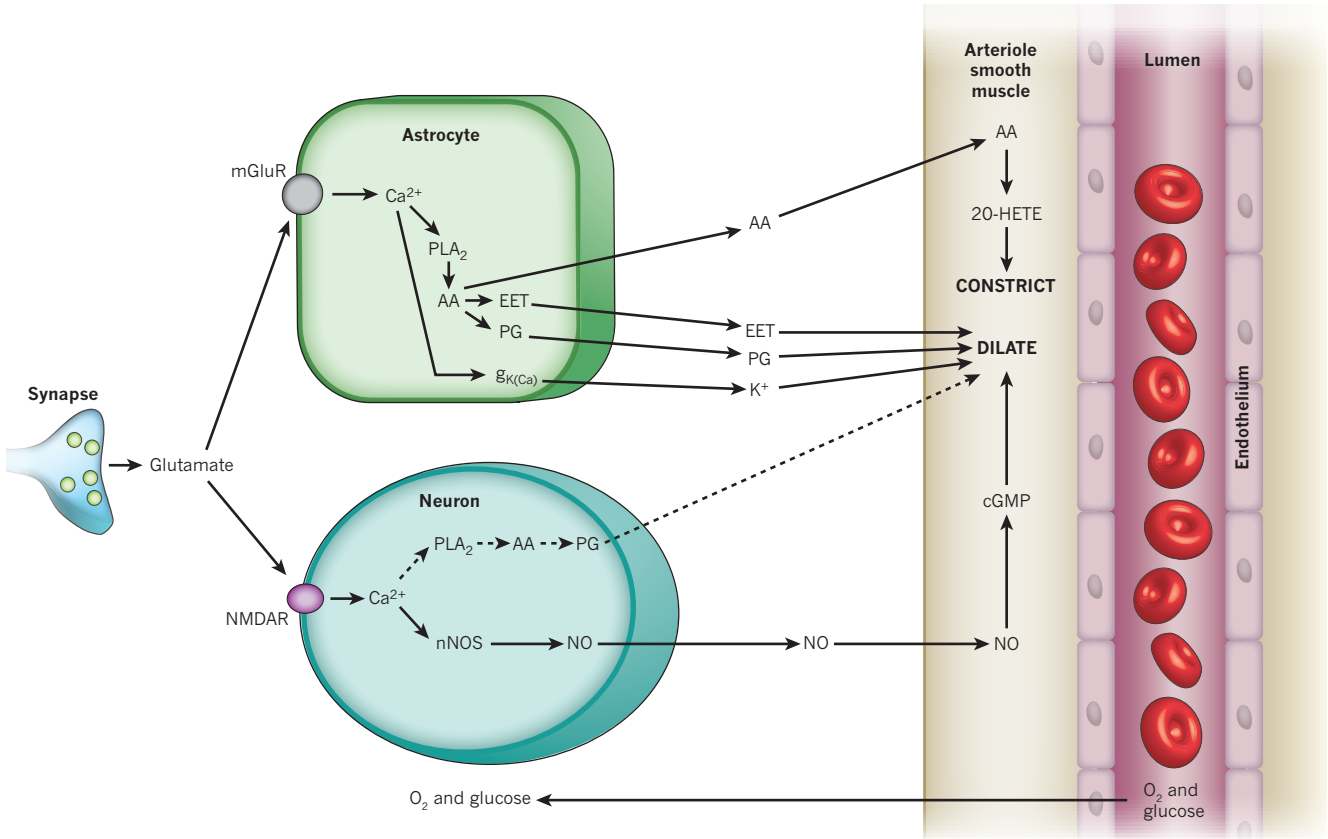

Figure 2 | Major pathways by which glutamate regulates cerebral blood flow. Pathways from astrocytes and neurons (left) that regulate blood flow by sending messengers (arrows) to influence the smooth muscle around the arterioles that supply oxygen and glucose to the cells (right, shown as the vessel lumen surrounded by endothelial cells and smooth muscle). In neurons, synaptically released glutamate acts on *N*-methyl-D-aspartate receptors (NMDAR) to raise $[Ca^{2+}]_i$, causing neuronal nitric oxide synthase (nNOS) to release NO, which activates smooth muscle guanylate cyclase. This generates cGMP to dilate vessels. Raised $[Ca^{2+}]_i$ may also (dashed line) generate arachidonic acid (AA) from phospholipase A_2 (PLA_2), which is converted by COX2 to prostaglandins (PG) that dilate vessels. Glutamate raises $[Ca^{2+}]_i$ in astrocytes by activating metabotropic glutamate receptors (mGluR), generating arachidonic acid and thus three types of metabolite: prostaglandins (by COX1/3, and COX2 in pathological situations) and EETs (by P450 epoxygenase) in astrocytes, which dilate vessels, and 20-HETE (by ω-hydroxylase) in smooth muscle, which constricts vessels. A rise of $[Ca^{2+}]_i$ in astrocyte endfeet may activate Ca^{2+}-gated K^+ channels ($g_{K(Ca)}$), releasing K^+, which also dilates vessels.

vasodilation. However, astrocytes may dilate vessels through a different K^+-based mechanism. When neuronal activity releases glutamate at synapses, some of the released glutamate escapes the synaptic cleft and activates metabotropic glutamate receptors (mGluRs) on astrocytes, thus increasing $[Ca^{2+}]_i$ in astrocytes[31] (Fig. 2). This increase in $[Ca^{2+}]_i$ has been reported[32] to lead to the opening of large-conductance Ca^{2+}-activated K^+ (BK) channels in astrocyte endfeet, releasing K^+ onto vessels. A caveat is that these experiments used a thromboxane analogue to maintain vessel tone in the brain slices studied, and this analogue has been shown to stimulate the trafficking of BK channels to the surface membrane of astrocytes[33]. It remains to be determined whether this K^+ release mechanism contributes to the regulation of blood flow *in vivo*.

There is strong evidence from brain slice and isolated retina preparations that astrocytes can control blood flow through the production and release of metabolites of arachidonic acid. When glutamate released from neurons activates astrocyte mGluRs (Fig. 2), the resultant rise in $[Ca^{2+}]_i$ activates phospholipase A_2, evoking the production of arachidonic acid from membrane phospholipids. The build-up of arachidonic acid leads, in turn, to the production of its metabolites, including prostaglandins and epoxyeicosatrienoic acids (EETs), which dilate nearby arterioles[15,34–37]. The prostaglandin involved is often suggested to be PGE_2, although many studies rely on inhibiting only the first enzyme (cyclooxygenase) in the prostaglandin-synthesis pathway, and other prostaglandins may contribute to the dilation. (The main pathways by which arachidonic acid derivatives are produced are shown in Fig. 3.) PGE_2 can relax vascular smooth muscle by binding to EP_4 prostaglandin receptors[38], which increase the activation of protein kinase A by cyclic AMP and thus decrease the phosphorylation of the myosin light chain[39]. The dilation produced by PGE_2 and other arachidonic acid metabolites also partly reflects their activation of K^+ channels in vascular smooth muscle cells[40,41], making the membrane potential more negative and thus decreasing the entry of Ca^{2+} through voltage-gated channels. EETs may also elicit dilation by inhibiting receptors for thromboxane[42], a vasoconstricting derivative of arachidonic acid. *In vivo*, a rise in $[Ca^{2+}]_i$ in cortical astrocytes results in a vasodilation that is partly (~70%) mediated by prostaglandins[43]. The rest of the dilation could be mediated by EETs[36], probably derived from astrocytes[37], although this remains to be shown directly.

In brain slices and in the isolated retina rises in $[Ca^{2+}]_i$ in astrocytes can also constrict vessels[34,44]. This is mediated[34] by the conversion of arachidonic acid to 20-hydroxy-eicosatetraenoic acid (20-HETE), probably by the enzyme CYP4A in vascular smooth muscle cells (Fig. 2). Whether a rise in astrocyte $[Ca^{2+}]_i$ causes dilation or constriction may in part be determined by the pre-existing tone of the vessel[45], but the O_2 concentration also has a key role in determining this (see 'O_2 modulates neurovascular signalling'). *In vivo*, a rise in astrocyte $[Ca^{2+}]_i$ produces vasoconstriction[46], mediated by a phospholipase A_2 derivative (presumably 20-HETE), during cortical spreading depression — a pathological wave of cell depolarization and refractoriness lasting minutes, after a brief period of increased excitability, which spreads across the cortex.

Modulation of these arachidonic acid metabolic pathways for therapeutic effect will depend on understanding the isoforms of the enzymes that synthesize the vasoactive messengers. For example, prostaglandins that mediate physiological vasodilation in response to a rise in astrocyte $[Ca^{2+}]_i$ are mainly produced by cyclooxygenase 1 (COX1)[43], which is

expressed in astrocyte endfeet[35], with a possible contribution by COX3 (ref. 47) or COX2 (refs 35, 48). However, in pathological conditions, expression of COX2 is upregulated in astrocytes[48] and this might also contribute to prostaglandin synthesis. Neurons also express COX2 and PGE synthase, and this may account for a COX2 component of functional hyperaemia seen in the cortex[49].

Another, conceptually distinct, pathway by which astrocytes may control cerebral blood flow involves neurotransmitter uptake. Although about half of the increase in blood flow that is induced by neural activity in the olfactory bulb is mediated by glutamate activating astrocyte mGluRs and releasing prostaglandins, a further one-third of the increase is mediated by the activation of glutamate transporters on astrocytes[50]. The glutamate transporter component did not involve a $[Ca^{2+}]_i$ rise or prostaglandin release, and still occurred when glutamate receptors were blocked. In a related study in the visual cortex, blocking glutamate uptake prevented astrocyte $[Ca^{2+}]_i$ increases and light-reflectance changes that are attributed to increased blood flow[51]. However, interpretation of these data is complicated because, first, blood flow was not measured directly, second, it was unclear whether the astrocyte $[Ca^{2+}]_i$ rises were generated by mGluRs or by glutamate uptake, and third, blocking uptake will raise the extracellular glutamate concentration, which could desensitize glutamate receptors and have indirect effects. Similarly, in the olfactory bulb, co-transport of Na^+ with GABA taken up into astrocytes raises $[Na^+]_i$. This in turn inhibits Na^+/Ca^{2+} exchange, raising $[Ca^{2+}]_i$ and constricting arterioles[52], presumably by releasing arachidonic acid to generate 20-HETE (Fig. 2), although this was not tested.

The importance of astrocytes versus neurons

The relative importance of the neuronal and astrocytic vasodilating pathways remains a matter of debate, because synaptic glutamate release raises $[Ca^{2+}]_i$ in both cell types[53], with some astrocytes showing a fast rise in $[Ca^{2+}]_i$, similar to that in neurons[54]. Blood flow responses are strongly correlated with local field potentials that reflect synaptic and action potentials in neurons[55,56], and ionotropic glutamate receptor antagonists significantly decrease both field potentials and blood flow responses to stimulation in the cerebellum, cortex and olfactory bulb[16,17,55]. This might indicate that a rise of $[Ca^{2+}]_i$ in neuronal dendrites initiates much of the increase in blood flow[53]. However, the glutamate release that generates field potentials also activates astrocyte mGluRs, so field-potential amplitudes will also be correlated with astrocyte receptor activation. A correlation of blood flow changes with field potentials is therefore not sufficient to prove that postsynaptic neuronal signalling dominates the control of blood flow. Furthermore, experiments blocking ionotropic glutamate receptors to prevent the release of neuronal messengers will decrease neuronal firing, inhibiting glutamate release onto astrocytes and thus preventing the release of astrocyte messengers. The potential for astrocytes to control blood flow is demonstrated by the fact that neural activity raises $[Ca^{2+}]$ in astrocytes[54,57], and that uncaging of Ca^{2+} in astrocytes dilates or constricts arterioles[34,35,43,44].

If the simplified scheme shown in Fig. 2 were correct, then the relative contributions of neuronal and glial signalling could be dissected by blocking either the neuronal NO pathway or the astrocyte arachidonic acid pathways. Knocking out or blocking nNOS (the only form of NOS contributing to functional hyperaemia[22]) does not affect whisker-stimulation-induced neural field potentials, but reduces the resulting blood flow increases by 37–60% in the somatosensory cortex[22,58]. In the cerebellum, knockout or inhibition of nNOS decreases activity-induced blood flow by 50–90%[13,24,25,59]. The remaining blood flow increase may be mediated by adenosine[14] or K^+ (ref. 60), but the possible contribution of prostaglandins and EETs has not been investigated. Blocking prostaglandin production with cyclooxygenase inhibitors reduces functional hyperaemia in the cortex[61,62] by 48–60% without affecting neuronal field potentials, whereas blocking EET production inhibits the response by 35–70%[36]. Thus, NO, cyclooxygenase and epoxygenase products are all important for generating functional hyperaemia in the cortex, whereas NO is more dominant in the cerebellum[59].

However, we will see below (see 'NO modulates astrocyte signalling') that the different pathways interact at the level of the enzymes producing arachidonic acid derivatives. In addition, a saturating interaction at the level of the arteriole smooth muscle membrane potential is expected if EETs and prostaglandins both dilate arterioles by opening K^+ channels, because once one pathway has hyperpolarized the cell out of the activation range of voltage-gated Ca^{2+} channels, then further opening of K^+ channels will have no effect. Caution is therefore needed in interpreting the results of experiments in which single enzymes are blocked.

Pathway-specific signalling differences

Even if functional hyperaemia is largely driven by synaptic glutamate release, it does not follow that all excitatory synapses are equally influential in controlling blood flow, even if they impinge on cells in the same

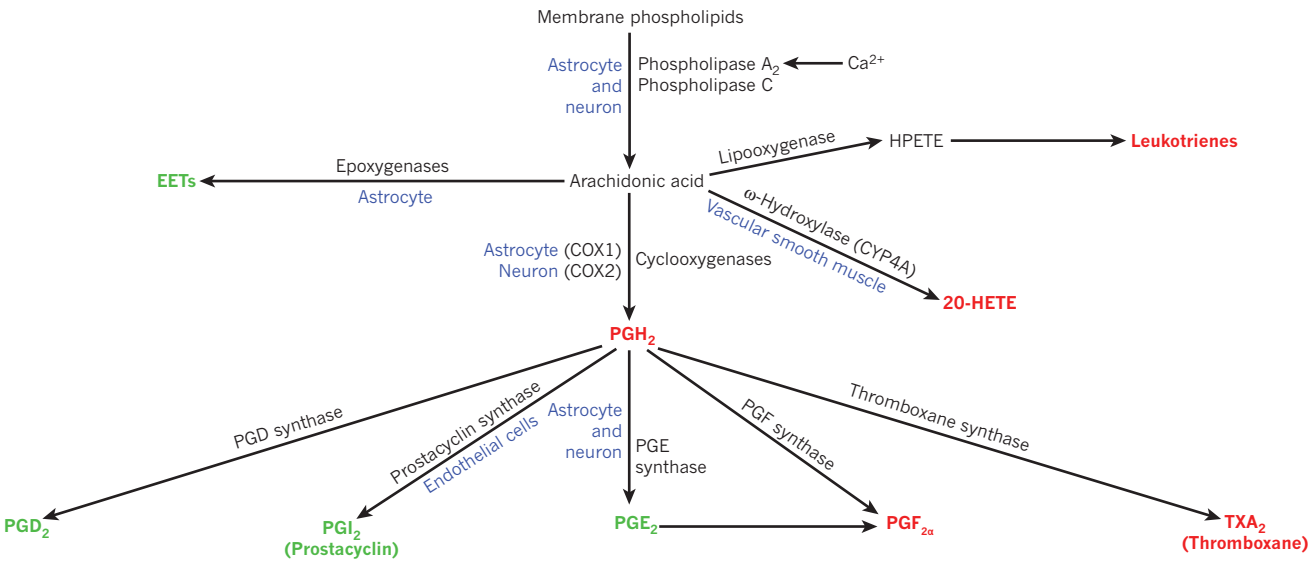

Figure 3 | Arachidonic acid metabolites that may contribute to control of cerebral blood flow. Arachidonic acid is formed from membrane phospholipids by Ca^{2+}-dependent and Ca^{2+}-independent lipases. Metabolites shown in green are vasodilators, red metabolites are vasoconstrictors, and blue denotes the location of some of the relevant enzymes. COX, cyclooxygenase; CYP, cytochrome P450 superfamily of enzymes; EET, epoxyeicosatrienoic acid; 20-HETE, 20-hydroxyeicosatetraenoic acid; HPETE, hydroperoxy-eicosatetraenoic acid.

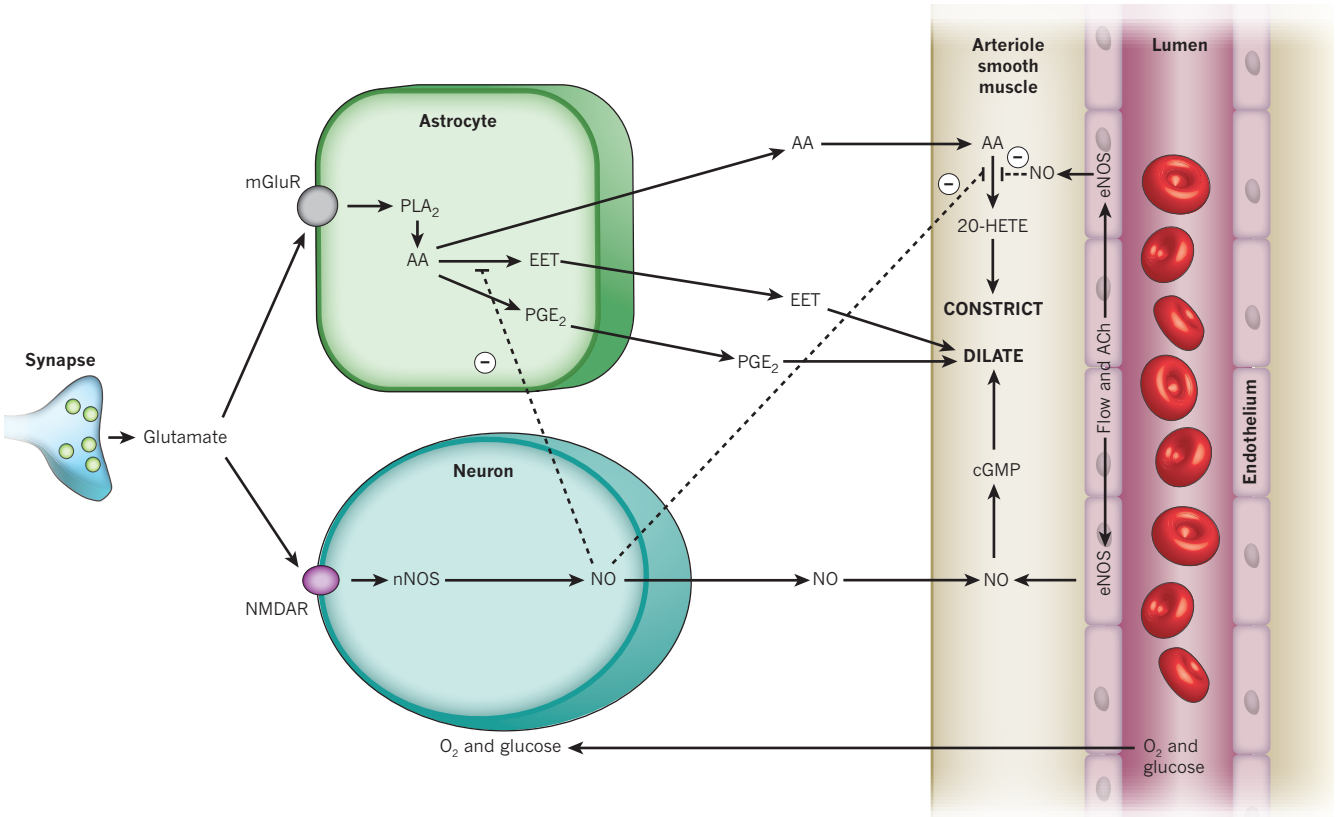

Figure 4 | Nitric oxide inhibits the production of key arachidonic acid-derived messengers. NO inhibits (dashed lines) the production of both the vasoconstricting 20-HETE and the vasodilating EETs[36,69]. NO also weakly stimulates COX1 and inhibits COX2 (not shown)[65]. Endothelial nitric oxide synthase (eNOS) can be activated by flow-induced shear stress or by acetylcholine (ACh). Other abbreviations as in earlier figures.

area. Indeed, activation of thalamocortical or transcallosal corticocortical inputs to the somatosensory cortex induces cerebral blood flow increases with a different dependence on the level of input activity[56], monotonically increasing with activity for the transcallosal pathway and showing a maximum at an intermediate level of input for the thalamocortical input. These differences may be partly due to differential activation of interneurons that release vasoactive peptides[56]. However, they may also reflect differences in the functional anatomy of neuro-vascular coupling for the two input pathways. For example, some of the 8,000 synapses innervating a typical rodent neocortical pyramidal cell[1] will release NO closer to arterioles, or induce a $[Ca^{2+}]_i$ rise in astrocytes that is more effective at releasing vasodilatory messengers onto arterioles, than will other synapses. Similarly, in the cerebellar cortex, although cerebral blood flow increases evoked by activation of the parallel and climbing fibre inputs to Purkinje cells depend partly on NO, the blood flow response to parallel fibre stimulation also involves a rise in $[K^+]_o$, whereas the response to climbing fibre activation partly involves adenosine release[60,63].

Thus, the relative importance of the neuronal and astrocyte pathways is likely to differ across brain areas, and even between different neural pathways in the same area. Consequently, the dependence of blood flow increases on the underlying neural activity will differ between different pathways, implying that functional imaging signals arising from these pathways will also reflect different aspects of neuronal function (see 'What does functional imaging measure?'). Similarly, therapeutic modulation of the signalling pathways controlling cerebral blood flow may have different effects in different brain areas, and on different neuronal pathways in the same area.

NO modulates astrocyte signalling

Assessing the relative significance of, or interfering therapeutically with, the different signalling pathways producing functional hyperaemia is complicated by interactions between them. The effects of the NO, cyclooxygenase and epoxygenase pathways can occlude each other. In the cortex, for example, functional hyperaemia is inhibited ~50% by blocking NOS and ~50% by blocking cyclooxygenase, but blocking both produces only a 70% inhibition[62]. Similarly, blocking NOS, blocking EET production, or blocking both pathways together all produce a ~60% inhibition of functional hyperaemia[37]. These results may be explained by interactions between the NO and arachidonic acid pathways, which are shown in Fig. 4.

Nitric oxide inhibits the enzymes that synthesize 20-HETE and EETs[64]. It also, much more weakly, inhibits prostaglandin production by COX2, but stimulates COX1 (ref. 65). The inhibition of the synthesis of the vasoconstrictor 20-HETE by NO is of particular interest for two reasons. First, this effect may underlie a significant fraction of the dilating effect of NO[66] (20–60% in different vessels[64]), which is independent of the NO vasodilation mediated by cyclic GMP (Fig. 4). Second, it provides a probable explanation for why, in the neocortex, NO is required for functional hyperaemia to occur, but is not the primary mediator of the increase in blood flow[23]. If the main cause of the blood flow increase is the generation of arachidonic acid by astrocytes, then having NO present to inhibit 20-HETE formation will ensure that only the vasodilatory prostaglandin and EET derivatives of the arachidonic acid will affect arteriole diameter. In the retina, however, where arteriole vasodilations are produced by EETs[34], the presence of NO promotes light-induced constrictions and inhibits dilations, suggesting that the production of EETs is more NO sensitive than is the production of 20-HETE[34].

O_2 modulates neurovascular signalling

Variations in O_2 concentration in brain tissue alter neurovascular coupling in two ways. The O_2 level affects the synthesis of the glial and neuronal messengers involved, and also alters the levels of lactate and adenosine that modulate the pathways by which these messengers

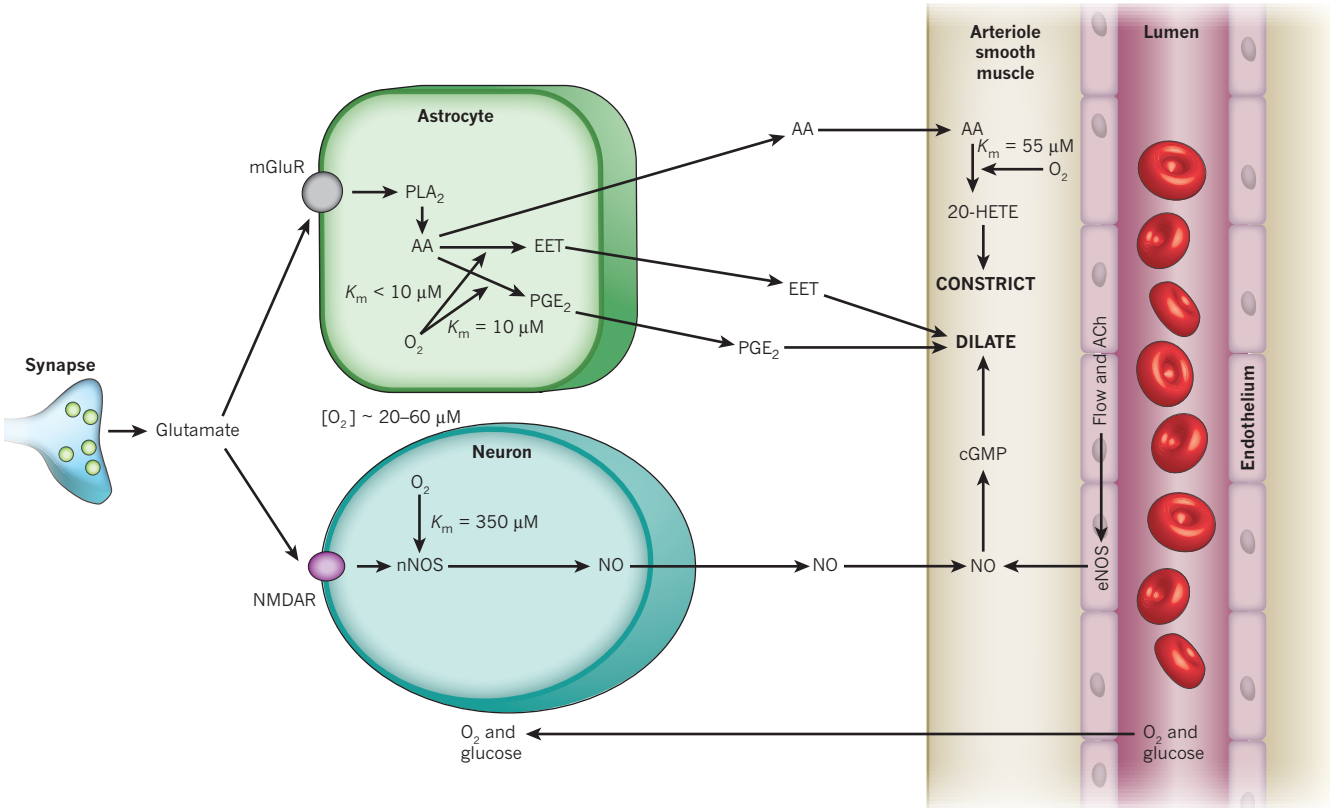

Figure 5 | Oxygen differentially affects the synthesis of neurovascular messengers. The concentration of O_2 in the extracellular space is 20–60 μM. This is significantly higher than the effective K_m for O_2 activating the enzymes synthesizing EETs and prostaglandins, but is in a range in which changes in O_2 concentration will modulate the production of NO and 20-HETE. Abbreviations as in earlier figures.

regulate vascular tone.

O_2 is needed for the synthesis of nitric oxide and the vasoactive messengers derived from arachidonic acid. Comparing *in situ* O_2 levels with the O_2 affinities of the O_2-sensitive reactions (Fig. 5) suggests how changes in O_2 concentration will affect neurovascular coupling. As O_2 concentrations are decreased, the synthesis of NO by neurons is expected to be inhibited first (Michaelis constant (K_m) ~ 350 μM at 25 °C[67]), followed by 20-HETE synthesis (K_m ~ 55 μM at 37 °C[68]), whereas EET synthesis (K_m < 10 μM at 37 °C[68]) and prostaglandin synthesis (K_m ~ 10 μM at 24 °C[69]) should be maintained at much lower O_2 concentrations. How do these values compare with the O_2 levels found in brain tissue? Neurophysiologists often use 95–100% O_2 (~1 mM, or 760 mm Hg) in solutions superperfusing isolated tissue, which lead to O_2 concentrations of ~100–150 μM in brain slices. This is much higher than the *in vivo* values[18,19] of 20–60 μM, which can even decrease by a further 13 μM during intense synaptic activity[18,19]. These *in vivo* values, which can be mimicked by superfusing brain slices with solution equilibrated with 20% O_2 (ref. 70), are comparable to or lower than the K_m values for O_2 in the synthesis of 20-HETE and NO. Thus, at *in vivo* levels of O_2, we expect NO and 20-HETE synthesis to be significantly limited by the amount of O_2 available.

Consistent with this view, lowering the O_2 concentration in solution superfusing brain slices from 95% to 20% (to lower the tissue $[O_2]$ from about 125 to 40 μM) has a dramatic effect on the vascular responses produced by uncaging Ca^{2+} in astrocytes[35]. With a supraphysiological O_2 concentration in the tissue, raising astrocyte $[Ca^{2+}]_i$ led to arteriolar constriction, whereas with a physiological $[O_2]$, vasodilation occurred. These changes are, in part, predicted from the modulatory effects of O_2 and NO on the signalling pathways outlined above (in this and the preceding section). The suppression of 20-HETE formation by the lower O_2 concentration is expected to reduce the vasoconstriction produced when arachidonic acid is generated in astrocytes (Fig. 5), and the lower $[O_2]$ will also result in less NO being present to inhibit the formation of vasodilatory EETs (Fig. 4) in tissues where these contribute to dilation.

However, two other factors were also shown to contribute significantly to the effects of lowering $[O_2]$ (ref. 35; Fig. 6). As O_2 concentrations decrease, the lack of energy for ATP synthesis causes an increase in the level of extracellular adenosine (Fig. 6), which binds to adenosine A_{2A} receptors on vascular smooth muscle cells to depress vessel constriction. In addition, a decrease in the rate of oxidative phosphorylation relative to the rate of glycolysis results in lactate production (Fig. 6). Monocarboxylate transporters release the lactate into the extracellular space, where it reduces the clearance of extracellular PGE_2 by the prostaglandin transporter (Fig. 6). Thus, when PGE_2 is released from astrocytes, extracellular PGE_2 increases to a greater degree, resulting in larger arteriole dilations[35]. This effect of lactate may partly explain why, in humans and rats *in vivo*, cerebral blood flow is regulated by the lactate/pyruvate concentration ratio and thus by the NADH/NAD^+ ratio[12]. Interestingly, lactate is released into the extracellular space during synaptic activity[71], which should promote vasodilation.

Despite our understanding of how O_2 levels regulate astrocyte-mediated neurovascular coupling in brain slices[35], imposing artificially high O_2 concentrations *in vivo* does not lead to smaller vasodilations or the emergence of vasoconstrictions[7]. This could reflect a potentiation of NO-mediated signalling from neurons to arterioles (relative to that occurring in brain slices superfused with 95% O_2) by the high O_2 level produced by hyperbaric O_2 (ref. 7) (as NOS activity is potentiated by the higher $[O_2]$; Fig. 5), which outweighs the effect of O_2 on astrocyte-mediated signalling.

Control of blood flow at the capillary level

Until recently, it was assumed that neurovascular coupling is mediated solely by changes in the tone of the smooth muscle that forms a continuous layer around arterioles. This idea has been challenged by

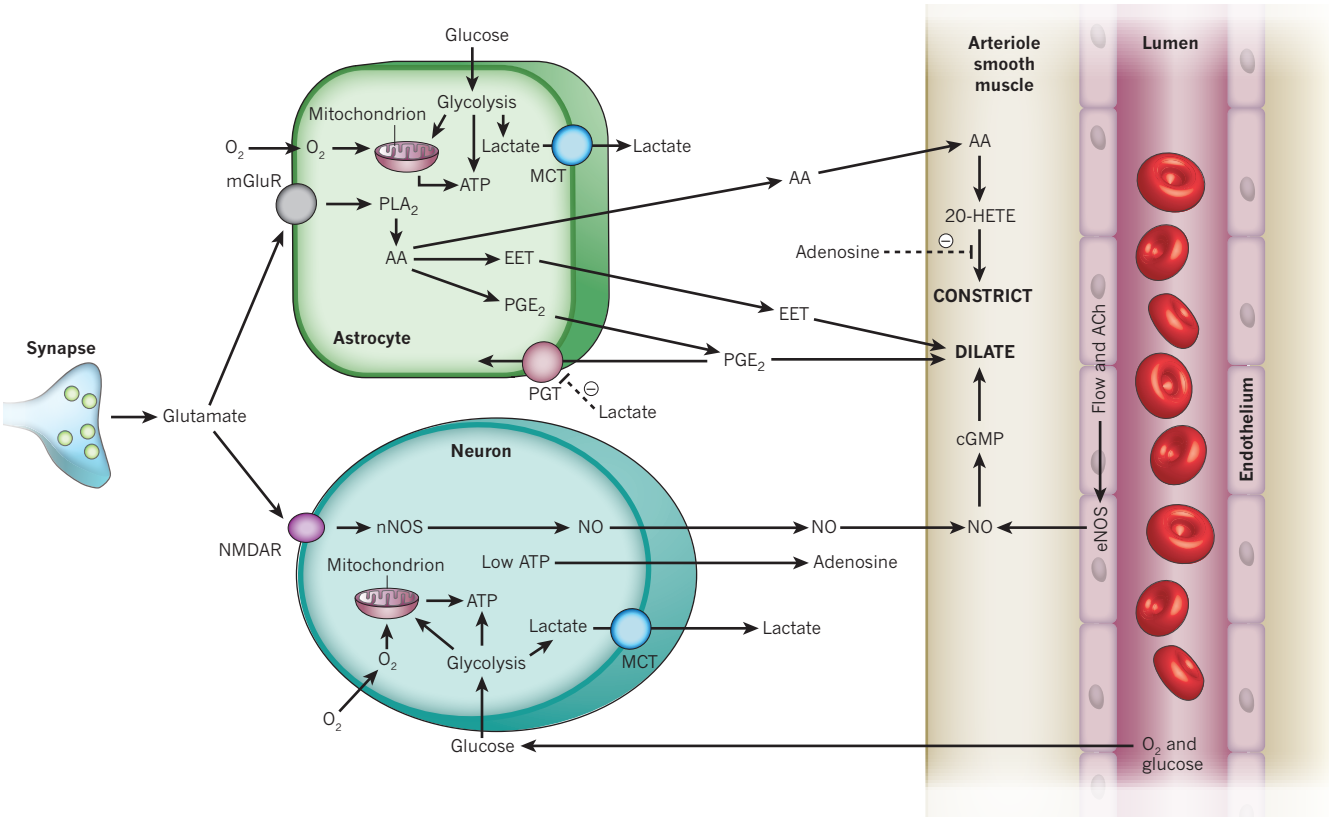

Figure 6 | Lactate and adenosine affect neurovascular signalling at low [O_2]. Low O_2 concentrations lead to mitochondrial oxidative phosphorylation failing to consume all the pyruvate produced by glycolysis, resulting in an export of lactate by monocarboxylate transporters (MCTs). Extracellular lactate inhibits the reuptake of PGE_2 by the prostaglandin transporter (PGT), promoting vasodilation[35]. Low energy levels also lead to the formation of adenosine, which inhibits 20-HETE-mediated arteriolar constriction by acting on adenosine A_{2A} receptors[35]. Other abbreviations as in earlier figures.

the discovery that pericytes — cells present at ~50-μm intervals along capillaries — can markedly alter capillary diameter, and thus potentially regulate cerebral blood flow at the capillary level.

Pericytes express contractile proteins, and their location on capillaries led to the suggestion, some 130 years ago, that they could constrict the microvasculature[72]. This idea was reinforced with observations that substances that alter arteriole diameter, including arachidonic acid derivatives and neurotransmitters, can contract and relax pericytes cultured on rubber membranes[73]. Importantly, in a series of papers, pericytes on isolated retinal capillaries were demonstrated to constrict or dilate in response to neurotransmitters, as a result of $[Ca^{2+}]_i$ alterations[74]. It is assumed that the signalling pathways controlling pericyte constriction and dilation will be similar to those for arterioles shown in Fig. 2, but this still needs to be tested. *In situ* in brain slices, pericytes constrict in response to noradrenaline and dilate in response to glutamate[75], and in the isolated retina, blocking ionotropic GABA receptors constricts capillaries, demonstrating that endogenous transmitter release can regulate capillary diameter[75]. Regulation of cerebral blood flow at the capillary level has yet to be demonstrated *in vivo* in physiological conditions[72], although it does occur after ischaemia[76].

Signals for contraction (presumed to be depolarization) and perhaps for dilation (hyperpolarization) can propagate from one pericyte to another[74,75]. This signal spread may occur through gap junctions between the interdigitating processes of the pericytes themselves, or through gap junctions with endothelial cells. Because active neurons are, on average, closer to pericytes than to arterioles[77] (8–23 μm away versus 70–160 μm), this signal spread raises the theoretical possibility that vascular responses to changes in neuronal activity may be initiated by pericytes and then propagated to upstream arterioles.

The importance of capillary diameter control by pericytes for regulating cerebral blood flow will depend on the fraction of the haemodynamic resistance of the vascular network that the capillaries contribute. Models in the literature[78,79] suggest that the capillary resistance is between 16% and 70% of the total vascular resistance (arterioles plus capillaries plus venules) within the brain parenchyma. If blood flow in capillaries obeys Poiseuille's law of fluid dynamics (see below), with flow proportional to the fourth power of the vessel diameter, then these values lead to the conclusion that a 2.1-fold capillary dilation produced by superfused glutamate in the presence of noradrenaline[75] could, in principle, increase blood flow 1.18- to 2.98-fold[72], and thus contribute significantly to functional hyperaemia. However, this may overestimate the role of pericytes if endogenous noradrenaline release is less than that applied when probing the effect of glutamate[75], or if the effect of superfused glutamate in brain slices overestimates dilations produced in perfused vessels *in vivo* by neurons releasing glutamate. On the other hand, Poiseuille's law severely underestimates the power of pericytes to regulate blood flow, because red and white blood cells have to deform considerably to pass through capillaries that constrict below ~5 μm — or they may not be able to pass through at all, which becomes important when pericytes constrict capillaries after ischaemia[76]. This will result in a more non-linear dependence of flow on diameter, enhancing the influence of capillaries on blood flow. Experiments are needed to define the importance of pericytes for regulating blood flow *in vivo*.

Blood flow rises more than energy use

Traditional analyses of sensory systems assumed that external stimuli induce action potentials in neurons with receptive fields 'tuned' to particular features of the stimuli. On this basis, low levels of spontaneous neuronal activity merely provide a 'noise' background against which the stimulus must be detected, and sensory input should greatly increase neuronal activity and hence cortical energy use. Recently, however, it has been suggested that incoming sensory information produces only small changes to the ongoing activity level in cortical neurons[80]. Indeed, across a range of sensory systems, less than a 10% change of neuronal

spiking is produced by perceptual tasks used in functional imaging experiments[81], and as a result sensory input alters cortical energy use by a relatively small fraction[82].

The fractional increase in blood flow induced by sustained neuronal activity is at least 4-fold greater than the increase in ATP consumption by the neurons[82]. This is consistent with blood flow being mainly regulated by feedforward neurotransmitter-mediated mechanisms rather than by a negative-feedback loop driven by energy demand (Fig. 1b, c), because a negative-feedback system could not produce a sustained increase in energy supply that is larger than the increase in energy consumption. As explained below (see 'What does functional imaging measure?'), this disproportionate increase in blood flow is the basis of the blood-oxygen-level dependent (BOLD) functional imaging technique. It was initially thought that most of the energy used when neurons are activated came from glycolysis rather than oxidative phosphorylation, because neuronal activity increases glucose uptake much more than O_2 uptake[83]. However, later studies found less of a difference in the glucose and O_2 uptake rates[84], and far more ATP is produced by oxidative phosphorylation than by glycolysis per glucose molecule. As a result, even with a much smaller increase in O_2 uptake than in glucose uptake[83], at least 60% of the extra ATP production would be occurring by oxidative phosphorylation[85], and more recent work suggests that almost all of the ATP is generated by oxidative metabolism of glucose[82].

A possible explanation for the large increase in blood flow induced by activity, relative to the increase in O_2 uptake and ATP production, was based on modelling of O_2 uptake: a large increase in blood flow (driven by glutamate release, not by O_2 deficit) may be needed to generate even a small increase in O_2 uptake[86]. However, pharmacologically blocking most of the increase in blood flow produced by activity has little effect on the increase in O_2 consumption[87], arguing against this explanation. At present, therefore, it seems that a large increase in blood flow (and glucose uptake) should not be needed to maintain energy supply during normal brain activity. Perhaps this large increase occurs as a by-product of a system that attempts to maintain blood flow during conditions of greater energy need that can occur pathologically (see 'Spreading depression and neurovascular coupling').

What does functional imaging measure?

BOLD functional magnetic resonance imaging (fMRI) of brain activity depends crucially on functional hyperaemia[88]. This technique detects a magnetic resonance radio signal emitted from proton spins. The strength of the signal is decreased by deoxyhaemoglobin, which is paramagnetic and makes the magnetic field less uniform. Without functional hyperaemia, the O_2 consumption powering neuronal activity would increase the deoxyhaemoglobin level and thus decrease the MRI signal. In fact, BOLD images show an increase in intensity in active brain areas. This is because the increase in blood flow evoked by neuronal activity brings in excess oxygenated blood, so that the deoxyhaemoglobin level falls and the MRI signal is increased. Thus, simplistically, the size of the BOLD fMRI signal is determined by the difference between the amount of functional hyperaemia, which increases the signal, and the use of O_2 by neurons, which reduces the signal (there is also a dependence on blood volume, which increases with the pressure increase driving the increased flow, which is ignored for simplicity here).

Because the effect of functional hyperaemia dominates, it follows that, to a large extent, BOLD fMRI signals reflect the causes of functional hyperaemia. When it was thought that activity-evoked blood flow increases were driven by energy use, fMRI images were believed to reflect energy use by active neurons. The discovery that functional hyperaemia is driven largely by glutamate release indicates, instead, that fMRI mainly images the neurovascular signalling consequences of synaptic activity[89]. Consistent with this, cortical BOLD signals correlate slightly better with field potentials reflecting stimulus-evoked synaptic currents than with stimulus-evoked action potentials[90], but they still correlate well with action potential activity[90] because cortical synaptic potentials are themselves well correlated with pyramidal cell spiking. It would be instructive to repeat this experiment in the cerebellum, where parallel fibre activity leads to a glutamate-mediated increase in blood flow, but to a decrease in principal (Purkinje) cell firing due to disynaptic inhibition[55].

Because BOLD signals mainly reflect the causes of functional hyperaemia, if we assume that this is largely produced by astrocyte signalling, BOLD fMRI images will essentially reflect the activation of astrocyte mGluRs (Fig. 2). Conversely, if the neuronal NO pathway dominates, then BOLD essentially images the activation of postsynaptic nNOS. But BOLD may still give information on neuronal spiking activity, for two reasons. First, glial $[Ca^{2+}]_i$ increases (and downstream generation of arachidonic acid derivatives) can be closely correlated with the activity of adjacent neurons[51], presumably because the glia respond to glutamate released at synapses onto the neurons. Second, typical voxel sizes used in fMRI (~1.5–3 mm) include many output synapses from the local axon collaterals of pyramidal cells in the cortex[91], and so the BOLD signal obtained, although originating from functional hyperaemia evoked by glutamate release, could nevertheless reflect the local pyramidal cell spiking activity that drives glutamate release at those synapses. For this to be the case, the effect on the vasculature of the axon collateral synapses would have to outweigh the effect of synapses carrying input to that part of the cortex from other brain areas.

The spatial resolution of BOLD fMRI has increased with the field strength used for imaging, and now approaches 200 μm in animals[85]. Even higher resolution can be obtained by optical imaging of changes in the oxy- and deoxyhaemoglobin concentrations within vessels[92]. The different spatial ranges of the vasodilatory mechanisms outlined in Fig. 2, that is, the distance that NO[93] or arachidonic acid derivatives can diffuse and the distance that $[Ca^{2+}]_i$ increases propagate through astrocytes, will limit the spatial resolution that functional imaging can potentially attain.

Spreading depression and neurovascular coupling

Functional hyperaemia could be particularly important for preventing neuronal death in pathological conditions in which energy use is raised, but it is in precisely these conditions that neurovascular coupling sometimes fails. One example is in cortical spreading depression, which occurs in migraine[94], when it is relatively benign, and repeatedly after stroke, subarachnoid haemorrhage or brain trauma, when it is associated with delayed neurological deficits[95–97]. Spreading depression is associated with a notable failure of brain ion homeostasis[98]. The extracellular potassium concentration rises transiently from ~3 to ~50 mM, depolarizing neurons and astrocytes, and the extracellular glutamate concentration rises[99] as a result of release from neurons and impairment of uptake[100]. Brain energy use increases[101], presumably because the increase in glutamate concentration activates an influx of Na^+ and Ca^{2+} ions that need to be pumped out, and the sodium pump is further activated by the rise in $[K^+]_o$. As a result, the cortex consumes more O_2 and glucose than is provided by the blood, resulting in a decrease in their extracellular concentrations[101–103]. This increase in energy use is pronounced during the first few minutes of spreading depression, but persists at a lower level for hours[101].

A failure of neurovascular coupling to provide a sufficient increase in blood flow during the increased energy consumption generated by spreading depression may underlie the neurological deficits associated with spreading depression after subarachnoid haemorrhage[97]. During spreading depression, cerebral blood flow is initially transiently increased[101,102] (less so in migraine than in stroke or brain trauma[94,104]), sometimes with a preceding decrease in blood flow[46,105]. After a few minutes, however, blood flow is reduced[94,101,102,105] by 20–30%. This decrease lasts for hours despite the increase in energy consumption during this period[101].

The rise in $[K^+]_o$ and associated cell depolarization during spreading depression raises $[Ca^{2+}]_i$ in neurons and astrocytes[46], leading to the release of messengers that evoke the observed blood flow changes. A release of calcitonin gene related peptide (CGRP) from trigeminal neurons innervating cerebral arteries, probably as a result of synaptic terminals depolarizing, contributes to the transient increase in blood

flow induced by spreading depression[104,106]. CGRP may act on receptors on smooth muscle cells or on astrocytes to produce this effect, although blocking the production of prostaglandins or EETs does not reduce the blood flow increase[104], indicating that a direct action on smooth muscle is more likely. Release of NO has also been suggested to contribute to the vasodilation[105,106], although this is controversial[104] and the effects of NOS blockers may reflect a need for NO to be present to suppress vasoconstriction mediated by 20-HETE (Fig. 4).

The sustained decrease in blood flow caused by spreading depression may be partly mediated by 20-HETE generated from astrocyte arachidonic acid. Reducing the increase in astrocyte $[Ca^{2+}]_i$ that accompanies spreading depression (using thapsigargin to prevent Ca^{2+} accumulation in internal stores), or blocking phospholipase A_2 to prevent arachidonic acid formation, both prevented the vasoconstriction[46]. This study, however, imaged only the early vasoconstriction occurring before the transient increase of blood flow, and not the long-lasting constriction that reduces blood flow for hours. During the prolonged constriction, normal functional hyperaemia is disrupted: vessels no longer dilate to physiological activation[107]. This is partly due to an impairment of the NO system[105,108], which normally dilates vessels directly or suppresses 20-HETE-mediated constrictions (Fig. 4), and may reflect the fall in O_2 concentration from ~24 to ~12 μM during this period[101], which will approximately halve NO production (Fig. 5). Indeed, more generally, the fact that O_2 supply by the blood depends partly on the release of the vasodilator NO, which requires O_2 for its formation, suggests a potentially damaging feedback loop. An increase in energy consumption lowers the O_2 concentration[101], which decreases NO formation, lowering blood flow and reducing the O_2 concentration further.

Neurovascular coupling after ischaemia

Following brain ischaemia, when an occluding intraluminal thrombus is cleared from a blood vessel either spontaneously or therapeutically using exogenous tissue plasminogen activator (tPA), after a brief period of hyperaemia there is a decrease in blood flow lasting several hours[2,109–111]. This inadequate matching of blood flow to neural activity may produce damage to neurons or glial cells beyond that caused by the initial ischaemia. The decreased flow, or 'no-reflow phenomenon', was attributed to a reduction in capillary diameter as a result of astrocyte endfoot swelling, causing capillary blockage by blood cells and fibrin[109], but later work suggested that this effect had been overestimated[112]. A failure of arteriole vasodilation mechanisms (tested with CO_2 and acetylcholine, which both release NO[113] and arachidonic acid derivatives[114]) may underlie the long-lasting decrease in blood flow[2,110,115]. However, in studies measuring blood flow and not vessel diameter, defects in arteriole dilation would be hard to distinguish from a situation in which arteriole dilation occurs but fails to increase blood flow because some capillaries are blocked.

Recent evidence indicates an important role for capillary pericytes in the long-lasting blood flow decrease following ischaemia. Some pericytes constrict at the start of ischaemia[75]. This may be because their $[Ca^{2+}]_i$ rises in the absence of ATP to pump Ca^{2+} out of the cell, although altered release of vasoactive messengers may also contribute (see below). Indeed, a decreased capillary diameter has been noted after ischaemia[111]. Interestingly, pericytes are also highly susceptible to damage in ischaemia[76], when ATP levels are expected to be low. This raises the possibility that pericytes constrict capillaries at the start of a stroke, and then stay in rigor (because no ATP is available to relax their contractile filaments), causing the capillaries to remain too small for the passage of red blood cells. In agreement with this, pericytes remain constricted and prevent red blood cell passage even two hours after re-opening an occluded parent artery[76]. Suppression of oxidative and nitrosative stress prevents this pericyte constriction, restores the patency of capillaries, and improves tissue recovery[76]. The involvement of oxidative or nitrosative stress suggests another mechanism by which pericytes might constrict. Peroxynitrite inhibits the formation of vasodilating prostacyclin by endothelial cells[116], resulting in the accumulation of vasoconstricting PGH_2 (Fig. 3), and may also inhibit the formation of vasodilatory EETs[117] and NO[118], any of which could lead to pericyte constriction.

The therapeutic use of tPA may itself alter neurovascular coupling, in part because it may extravasate and gain access to the brain parenchyma when the blood–brain barrier becomes leaky in ischaemia (a process that may be enhanced by tPA upregulating[119] matrix metalloprotease 9). There is debate about whether tPA is neurotoxic[119], but endogenous tPA, which is released from neurons, cleaves the NR1 subunit of NMDA receptors and thus enhances NMDA receptor signalling[120]. Independent of this effect, endogenous tPA is essential for neurovascular coupling mediated by NMDA receptors and NO, because it regulates the phosphorylation of nNOS and thereby promotes NO release[121]. Accordingly, exogenous tPA promotes vessel dilation in an NO-dependent manner[122], but it also enhances the failure of vasodilation that occurs after stroke[123]. To complicate the interpretation of these data, when used clinically tPA is co-packaged with a vehicle, a high concentration of L-arginine, which will promote NO formation by NOS. This will contribute to vasodilation and could possibly damage neurons.

Neurovascular coupling in Alzheimer's disease

There is increasing evidence for vascular factors having a causal role in the development of Alzheimer's disease[3]. Many patients with Alzheimer's disease have regional cerebral hypoperfusion[124], which correlates with cognitive decline[125]. Amyloid-β peptide decreases functional hyperaemia by promoting oxidative stress[126], which inhibits the production of astrocytic and neuronal vasodilating messengers[116–118]. A raised basal $[Ca^{2+}]_i$ and enhanced occurrence of spontaneous Ca^{2+} waves in the astrocytes of mice with amyloid-β plaques[127], together with increased contractility of vascular smooth muscle cells[128], will also disrupt the normal regulation of cerebral blood vessels by astrocytes and neurons. Such changes in neurovascular coupling may make it hard to interpret BOLD fMRI signals from older subjects[129], just as inhibiting neurovascular coupling pharmacologically alters the relationship between neuronal activity and BOLD signals[20]. Similarly, the formation of an astrocytic scar after traumatic brain injury is likely to profoundly alter neurovascular coupling.

Prospects for new therapies

There have been four major conceptual shifts in our understanding of how cerebral blood flow is regulated. It is now thought that neurotransmitters, particularly glutamate, rather than energy use are the principal agents generating activity-induced blood flow; that astrocytes mediate a large part of this blood flow control; that O_2 concentration regulates the relative importance of the signalling pathways involved; and that control of blood flow occurs at the capillary level as well as the arteriole level. These developments not only provide an increased understanding of how neural computation is powered, they also offer opportunities for developing therapies for treating disorders of cerebral blood flow.

In general, identifying the signalling pathways that regulate cerebral blood flow provides opportunities for manipulating those pathways therapeutically. This is conceptually straightforward for neurotransmitter-mediated signalling pathways that depend on well understood receptors and enzymes that can, in principle, be blocked. This could be done, for example, at the level of astrocyte mGluRs or the enzymes that generate vasoactive messengers such as cyclooxygenase, epoxygenase, CYP4A or NOS. The existence of many pathways regulating blood flow (Fig. 2) may allow subtle therapeutic modification of blood flow in different conditions or brain areas, so long as interactions between the different pathways (Fig. 4) are taken into account. The advent of new imaging techniques for rapid assessment of patients' cerebral blood flow could also enable therapies to be given earlier, before neuronal damage has been initiated. In particular, the signalling changes that occur in spreading depression and stroke suggest new therapeutic approaches, as follows.

The decrease in blood flow that follows spreading depression[101,102,105], which may contribute to cognitive decline after brain trauma, stroke

or vasospasm after subarachnoid haemorrhage[97], may be produced by excessive generation of vasoconstricting 20-HETE formed from astrocyte arachidonic acid. This could be tested using inhibitors of the CYP4A enzyme that generates 20-HETE. A role for pericyte constriction in this phenomenon should also be tested.

The long-lasting decrease in blood flow observed after experimental ischaemia[2,109–111], produced in part by pericyte constriction[75,76], indicates that even rapid dissolution of a clot using tPA may be limited in its effects on restoring perfusion. This suggests that, in combination with tPA application, it would be worth trying to preserve normal pericyte function. Pericyte constriction and death after ischaemia are mediated by oxidative or nitrosative stress[76], so infusing antioxidants at the same time as tPA may be a useful adjuvant approach. Preclinical studies assessing how pericyte properties vary with age and gender, and whether pericyte contraction contributes to pathology in hypertension (which is associated with neurovascular dysfunction[3]) or hyperglycaemia[72,74,130], will also be valuable. ■

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